Advance Journal of Pharmaceutical Research & Review
Volume 2, Issue 5, May 2025, PP: 01-08, ISSN No: 3048-491X

# Comprehensive Analysis of Phytochemical Constituents, Antioxidant Activities and Antimicrobial Studies of Methanolic Extract of Syzygium Cumini Leaves.

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Date of Submission: 04-05-2025 Date of Acceptance: 17-05-2025

**ABSTRACT**
Syzygium cumini also known as Jambolan, is one of the commonly used medicinal plants for treating a variety of ailments, especially diabetes. The present study is to provide phytochemical constituents, antioxidant, proximate of S. cumini (L). Since the plant was first made commercially available several decades ago, it has been thought of as an antidiabetic plant. This natural medicine's goal is to affect the bodily systems that are influenced by the chemicals it contains. The current study adds to our understanding of the photochemical makeup of S. cumini and their antioxidant properties from a pharmacologic standpoint. Research on phytochemicals, antioxidants, proximate analysis confirms that S.cumini of methanolic extract contains carbohydrates, glycosides, flavonoids, phenols, tannins, saponins, proteins, and amino acids, in addition to having antioxidant potential**.**

**Keywords:** Syzygium cumini, DPPH, FRAP, antioxidant, phytochemicals, proximate composition.

## I. INTRODUCTION

Herbs of medicine are being used throughout the world, and these herbs are found to be the original source for most of the drugs. Medicinal plants contain so many bioactive compound that acts as therapeutic agents are used for the treatment of human disease. The perceived effectiveness, low frequency of severe side effects, and affordability of herbal medication appear to be its main advantages.

The regions with the highest levels of variety are Malaysia and northeastern Australia, where many species are poorly understood and many more lack taxonomic descriptions. Numerous fruits in this family have a long history of usage as both consumables and traditional medicines in various ethnobotanical practices across the tropical and subtropical world [1], and plants in this family are known to be rich in volatile oils that have been found to have medicinal applications [2]. Around the world, some of the edible Syzygium species are planted in tropical regions. Medicinal plants are a rich source of new medications that are used as lead molecules in synthetic drugs, pharmaceutical intermediates, nutraceuticals, food supplements, folk medicines, and traditional medical systems. For a wide range of illnesses, a sizable section of the global population, particularly in underdeveloped nations, relies on traditional medicine [3]. Eighty percent of people in several Asian and African nations receive their primary medical care from traditional medicine. Between 70% and 80% of people in many wealthy nations have utilized complementary or alternative medicine in some capacity [4]. While functional foods are fortified foods that closely resemble the original natural food, dietary supplements might be liquid concentrates or pills. Consuming food produced from plants has long been demonstrated to provide health benefits in traditional medical systems; this is mostly due to the phytochemical elements, such as polyphenols, that are found in some plants [5]. Syzygium cumini leaves steeped in alcohol are prescribed for diabetes, and they are also used to treat skin conditions.

There is some antibiotic activity in the bark, flower buds, leaves, and stems [6]. Antimicrobials are medications that kill microorganisms, stop them from growing or multiplying, or stop them from acting in a pathogenic manner [7]. A natural chemical that a microorganism produces to destroy another. Antibiotics are a broad and varied class of medications that work against illnesses by preventing bacteria from growing and reproducing. Antibiotics are a broad and varied class of medications that work against illnesses by preventing bacteria from growing and reproducing. Tetracyclines are effective against both gram-positive and gram-negative microorganisms, but penicillin is often employed against gram-positive bacteria [8]. Medicinal plants like aloe vera, turmeric, tulsi, pepper, elachi and ginger are commonly used for various kinds of diseases. Approximately 7000 different plant species have been used as food by the people. It is a good source of medicine even for life – threatening

diseases. Bioactive compounds such as carotenoids, polyphenols, saponins, are found in the plants and these act as secondary metabolites for the treatment of various disease.

Jamun is a huge evergreen tree that is well known in India. It is scientifically named as Eugenia jambolona or Syzygium cumini. The other common names of jamun are Indian blackberry, java plum, jambu, black plum, jambul, jam kol, brahaspati, gambu, jambool, kalajam, Malabar plum, naval, nerale beeja, neredu, pomposia, portuguese plum etc. India is a leading producer of jamun. Worldwide, total production of jamun is 13.5 million tonnes out of which India contributes about 15.4%.The leaves have a characteristic smell like turpentine, and are simple, dark green, opposite, oblong-oval or elliptical, glossy, smooth, leathery in touch and blunt or tapering at the apex point. Features of Trees: Height ranges from 10 to 20 meters (33 to 66 feet) in diameter of the trunk. falls between 30 and 60 cm (12 and 24 inches). Bark exhibits a smooth, grayish-brown appearance with a width of 2 to 5 millimeters (0.8 to 2 inches) and a length of 5 to 15 centimeters (2 to 6 inches). The entire margin has a wavy texture. The surface Smooth and glossy, with a light green underside and a dark green top surface. Characteristics of Flowers: At the end, the kind panicle is 5–10 cm (2–4 inches) long [9]. Dried leaves and bark are used to treat diabetes. Fluid extract of seeds is taken orally as an anti-inflammatory and hot water extract is used for antipyretic action. Fresh leaf juice is taken orally for blood pressure. Many traditional and oriental medicinal systems, including Chinese, Ayurvedic, and Unani medicine, use the seeds as a natural treatment for conditions like hyperglycemia, ulcers, dysentery, asthma, glycosuria, and bronchitis [10].

Stembark juice is taken for constipation and it is found useful to stop blood discharge in the feces when consumed mixing it with buttermilk. The leaves, steeped in alcohol, are prescribed in diabetes. The leaf juice is effective in the treatment of dysentery, either alone or in combination with the juice of mango or emblic leaves. Jambolan leaves may be helpful as poultices on skin diseases. They yield 12 to 13% tannin (by dry weight). Pharmacological actions of syzygium cumini has anti-allergic, antibacterial, anti-cancer, anticlastogenic, anti-diarrheal, anti-fertility, anti-hyperlipidemic, anti-inflammatory [11].

## II. MATERIALS AND METHODS

### 1. COLLECTION OF PLANT MATERIALS:

The fresh, mature leaves of *Syzygium cumini* were collected mainly in old town (OT) and around Cuddalore district, Tamil Nadu, India.

### 2. CHEMICALS:

DPPH, Sodium Nitroprusside, Sulphanalamide, Naphthylethylene diamine dihydrochloride, ferrous ammonium sulphate and all other chemicals used were of analytical grade.

### 3. PREPARATION OF *SYZYGIUM CUMINI* EXTRACTS

Plant material was washed thoroughly with tap water and shade dried for 2 weeks. Course the powder of leaves obtained by crushing the leaves in an electronic blender. Twenty gram of powdered leaves of *Syzygium cumini* were taken separately and extracted with 200ml of methanol, ethyl acetate and water separate in soxhlet apparatus at 37°C for 24 hours. The extracts were then evaporated under room temperature for 2 days. The dried extract was stored in air tight containers at 4°C for further studies.

### 4. PRELIMINARY PHYTOCHEMICAL SCREENING

The extract of *Syzygium cumini* leaves were subjected to preliminary phytochemical screening of various plant constituents.

**TEST FOR ALKALOIDS**

**Dragendorff" s Test:**

**Dragendorff" s Reagent:** eight grams of Bi(NO3)3 5H2O was dissolved in 20 ml of HNO3 and 2.72 g of potassium iodide in 50 ml of H2O. There were mixed and allowed to stand until KNO3 crystals formed. The supernatant was decanted off and made up to 100 ml with distilled water.

**Procedure:** 0.5 ml of the extract was added to 2 ml of HCL. To this acidic medium, 1 ml of Dragrndorff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

**Wagner's Test**

**Wagner's Reagent:** 1.2 g f iodide and 2.0 g f potassium iodide were dissolved in 5 ml of sulphuric acid and the solution was diluted to 100 ml.

**Procedure:** 10 ml of the extract was acidified by adding 1.5% V/V of HCL and a few drop of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

**Mayer's Test:**

**Mayer's Reagent:** 1.36 g of Mercuric Chloride was dissolved in 60 ml of distilled water and 5g of potassium iodide in 10 ml of water. The solution was mixed and diluted to 100 ml with distilled water.

**Procedure:** 1.2 ml of the extract was taken in a test tube, 0.2 ml of dilute HCL and 0.1f ml of Mayer's reagent were added. Formation of yellowish buff coloured precipitate confirmed the presence of alkaloids.

**TEST FOR Flavonoids**

**Shinoda's Test:** In a test tube containing 0.5 ml of extract, 5-10 drops of diluted HCL and a small piece of Zing Chloride or Magnesium were added and the

solution was boiled for few minutes. In the presence of flavonoids, reddish pink colour was produced.

**Alkaline Reagent Test:**
To 1 ml of the extract, a few drops of dilute sodium hydroxides were added. As intense yellow colour was produced in the plant extract, which becomes colourless on the addition of a few drops of dilute acid indicates the presence of flavonoids.

**TEST FOR CARBOHYDRATE**
A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.
**Molisch's Test**
The filtrate was treated with 2-3 drops of 1% alcoholic α-naphthol solution and 2 ml of concentrated H2SO4 was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.
**Benedict's Test**
Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.
**Fehling's Test**
Filtrate was hydrolysed with dil. HCL, neutralized with alkali and heated with Fehling's A &B solutions. Formation of red precipitate indicates the presences of reducing sugars.

**TEST FOR GLYCOSIDES**
The extract was hydrolyzed with HCL for few hours on a water bath and the hydrolysate was subjected to Legal's or Borntrager's Test to detect the presence of glycosides.
**Legal's Test**
To the hydrolysate, 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glucose-des.
**Borntrager's Test**
The hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute Ammonia solution was added. Ammonia layer acquired pink colour, shows the presence of glycosides.

**TEST FOR SAPONINS:**
The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of foam shows the presence of saponins
1 ml of the extract was treated with 1% Lead Acetate solution. Formation of white precipitate indicates the presence of saponins.

**TEST FOR TANNINS**
**Ferric Chloride Test:**
To 1-2 ml of extract, a few drops of 5% aqueous ferric solution was added. A violet colour formation indicates the presence of tannins.
**Lead acetate test:**
In a test tube containing about 5 ml of the extract, a few drops of 1% lead acetate was added. A yellow precipitate was formed, indicates the presence of Tannins. 5 ml of the extract was treated with 1 ml of 10% aqueous Potassium dichromate solution. Formation of yellowish brown precipitate suggests the presence of tannins.

**TEST FOR PROTEIN AND AMINO ACIDS**
**Ninhydrin Test**
1 ml of the extract was treated with few drops of Ninhydrin reagent. Appearance of purple colour shows the presence of amino acid.
**Biuret Test**: Equal volume of 5% NaOH solution and 1% Copper sulphate solution were added to 1 ml of the extract. Appearance of pink colour shows the presence of protein.

**TEST FOR PHENOLS**
A small quantity of the extract was treated with 1% aqueous or alcoholic Ferric chloride solution. Formation of green, purple, blue or black colour indicates the presence of phenol.
A small quantity of the extract was treated with aqueous mixture of 1% Ferric chloride and 1% Potassium Ferric cyanide. Appearance of green or purple or blue colour shows the presence of phenols.
**TEST FOR PHYTOSTEROLS**
**Salkowski's Test:** Extract were treated with chloroform and filtered. The filtrates were treated with few drops of Conc.Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
**Libermann Burchard's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

**DETERMINATION OF MOISTURE CONTENT**
An empty porcelain crucible with lid was heated well and cooled in a dessicator, heating, cooling and washing are repeated until a constant weight was obtained 3g of leaf powder was taken and heated in an oven at 100°C for 6 hours. The crucible was cooled in a dessicator and weighed again. This procedure was repeated until a constant weight was recorded.
The difference in weight of crucible leaf powder before and after heating was noted. Form that the percentage of moisture content was noted.

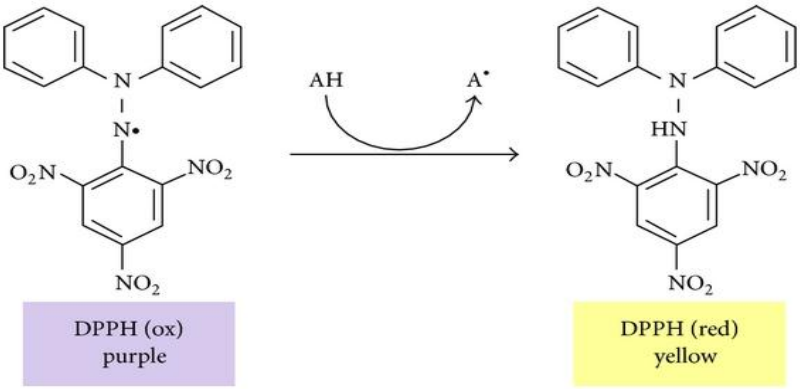
**DETERMINATION OF ASH CONTENT**

An empty porcelain crucible was heated in a bunsen burner. The crucible was cooled in a dessicator and weighted. 3g of leaf powder was weighed and heated until the sample gets charred, so that the organic matter is removed due to its high temperature. The crucible was placed in a muffle furnace till all the materials were completely charred at 60°C for 5-6 hours. The ash obtained will contain the local mineral content of thee food material where the sample was heated to 500-600°C the organic matter present in the food gets decomposed to Co2 and water. The remaining was then called as ash. Then the crucible was cooled and weighed accurately.

**ANTIOXIDANT ANALYSIS OF SYZYGIUM CUMINI**

The radical scavenging activity of methanolic extract of *S.Cumini* was determined by using DPPH assay according to the method [13]. Methanolic extract of *S.Cumini* at different concentrations (100-500µg/ml) was diluted with ethanol. 1 ml of 0.3mM DPPH in ethanol solution was added to the sample taken at different concentration and allowed to react at room temperature. Ethanol and DPPH were used as a blank.

BHT and Ascorbic acid were used as standard. After 30min the absorbance value were measured at 518nM and were calculated using the following formula.




2,2'-diphenyl-1-picrylhydrazyl 2,2'-diphenyl-1-picrylhydrazine

% inhibition = {A control-A test/A control X 100).

**2. NITRIC OXIDE RADICAL SCAVENGING ACTIVITY:**

Nitric oxide generated form sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction [14]. The reaction mixture (3ml) containing 10 mM sodium nitroprusside in phosphate buffered saline and the reference compound at different concentrations were incubated at 25°C for 150 min. A 0.5-ml aliquot of the incubated sample was removed at 30-min intervals and 0.5 ml Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihyrochloride in 2% $H_3PO_4$) was added. The absorbance of the chromophore formed was measured at 546nm. All tests were performed in triplicate. Percent inhibition of the nitric oxide generated was measured by comparing the absorbance values of control and test preparations.

NO Scavenged (%) = (A cont - A test)/A cont × 100

**3. Hydrogen Peroxide scavenging activity**

H2O2 content was estimated by following ferri-thiocyanate methods [15]. For H2O2 estimation, 0.1 ml of extract was made up to 1.6 ml with distilled water. Then 0.4 ml of trichloroacetic acid (TCA), 0.4ml of ferrous ammonium sulphate and 0.2 ml of potassium thiocynate was added to the extract. Absorbance of mixture was measured at 480 nm and compared to H2O2 standard. Peroxide content was expressed as µmol/ g FW.

**ANTIMICROBIAL ACTIVITY OF SYZYGIUMI CUMINI**

**Antibacterial activity**

Antibacterial activity of the leaf extract was determined by using agar well diffusion method. The antibacterial activity was done separately for 50 mg/ml and 100 mg/ml and it was prepared from the four concentrated extracts using distilled water and kept as test solution. The bacterial strains were sub cultured in 10 ml of nutrient broth and incubated at 37°C for hours. About 250 ml of nutrient agar was prepared and sterilized. It was then cooled and about 15 ml of nutrient agar was poured into pre-sterilized Petri plates. The agar was allowed to solidify. The bacterial cultures were spread over each plate by spread plate technique [16].

Four wells were formed on the agar plate using gel puncture. For standard, antibiotic Ciproflaxin is placed at the center of the petric plate. About 20µg of four extract were added into each well and incubated at 37°C for 24 hours. The zone inhibition was measured for each extract. In all cases six replicates were maintained. The mean diameter

of inhibition zone and standard duration were calculated.

**Antifungal activity**

The poison plate technique was used to investigate the antifungal activity of against fungal pathogens [15]. About 1% concentration of methanol leaf extract was prepared and mixed separately with potato dextrose agar medium and poured aseptically in sterilized Petri plates and similarly for Ethyl acetate. Acetone and aqueous. Control contains only the potato dextrose agar (PDA). The standard was also prepared by using ciproflaxin. A fungal organism was taken in the inoculation loop and it is spot inoculated at the center of the petri plate containing PDA agar. The growth of the fungal organisms was observed for 5 days. After 5 days of incubation, the growth of the organisms was measured and the present growth inhibition was calculated.

## III. RESULTS AND DISCUSSION

### I. PHYTOCHEMICAL SCREENING

**TABLE 1**

| S.No | Phytochemical test | Aqueous extract | Methanol extract | ethyl acetate extract |
|---|---|---|---|---|
| 1 | Alkaloids | - | - | - |
| 2 | Carbohydrates: | | | |
| | a.Molisch's test | + | + | + |
| | b.Benedicts test | + | + | - |
| | c.Fehling's test | + | + | - |
| 3 | Flavonoids | + | + | + |
| 4 | Glycosides | - | + | - |
| 5 | Saponins | - | - | - |
| 6 | Tannins | + | + | + |
| 7 | Protein and Amino Acids | + | + | + |
| 8 | Phenols | + | + | + |
| 9 | Phytosterols | - | - | - |

+ indicates positive (Present) - indicates negative (Negative)

### II. PROXIMAL ANALYSIS

**TABLE: 2**

| | |
|---|---|
| ASH | 2.21 ± 0.438 |
| MOISTURE | 3.24 ± 0.483 |

### III.ANTIOXIDANT ANALYSIS

**RESULT FOR DPPH SCAVENGING ACTIVITY**

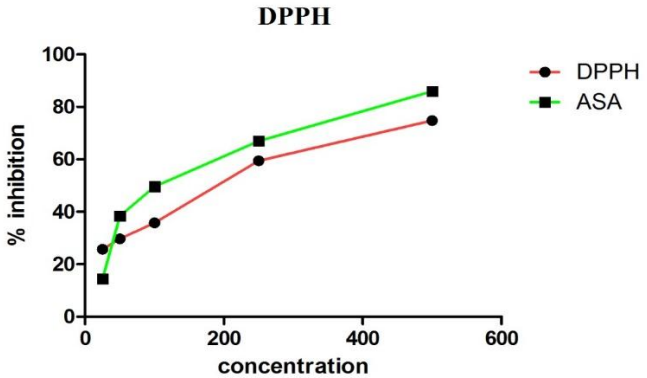



**Figure: 1**

**Nitric oxide scavenging activity**

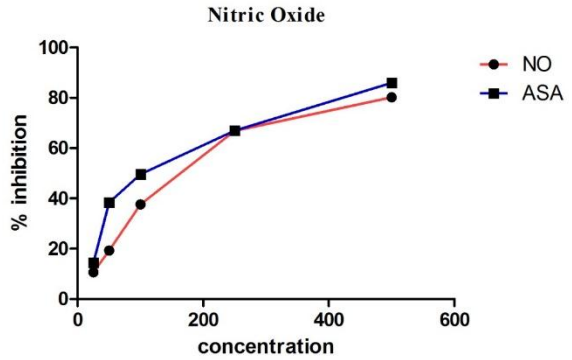



**Figure 2**

**Hydrogen peroxide scavenging activity**

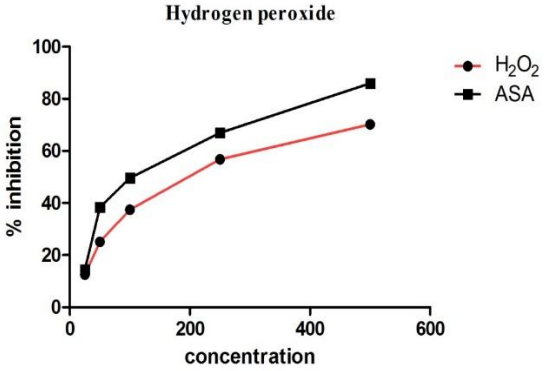
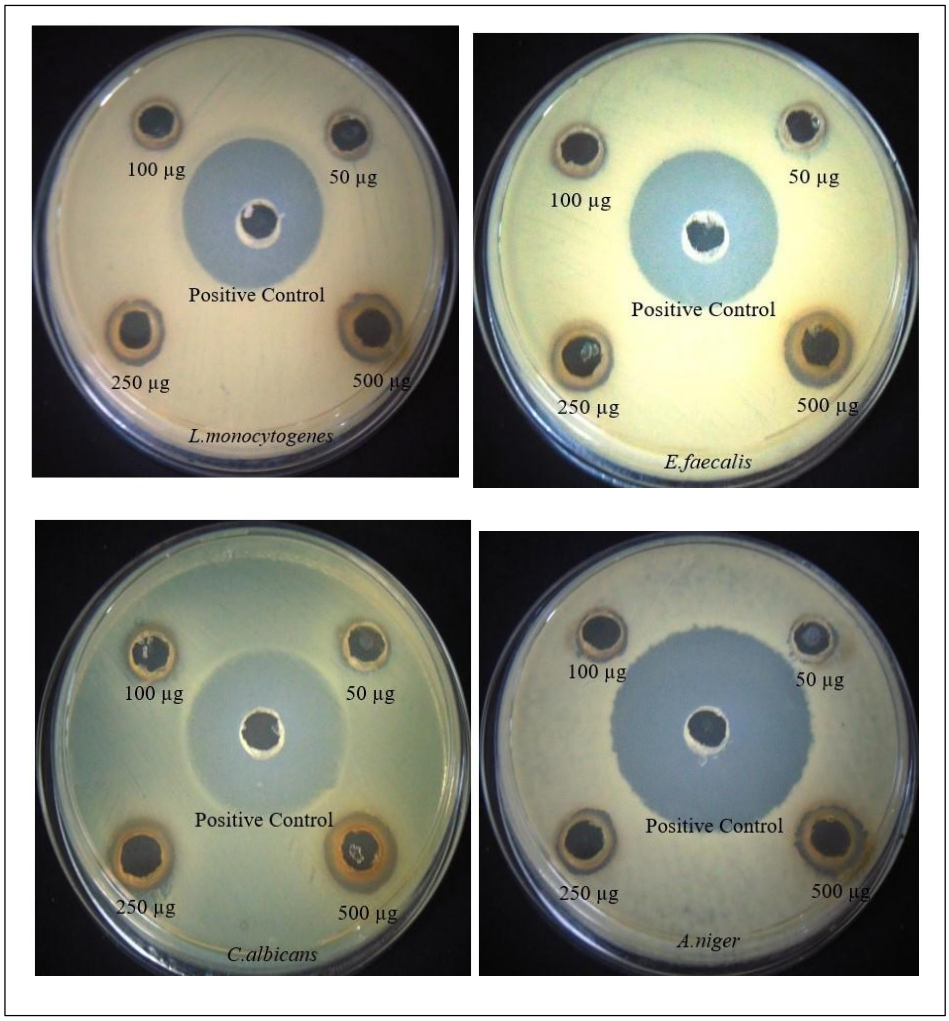



**Figure 3**

## IV.ANTIMICROBIAL ACTIVITY




## IV. DISCUSSION

The present study strengthens the pharmacologic knowledge of the phytochemical composition of S. cumini leaf and also its antibacterial, antimicrobial activities. As new drugs of plant origin are being developed it is appropriate to consider natural plants for the treatment of various diseases. The ability of the natural medicine to influence the body systems depends on the chemical composition that it contains.

**Preliminary Phytochemical Screening of methanolic extract of S. cumini leaves.**

**Table1:** reveals the qualitative phytochemical screening of the methanolic leaves extract of *S. Cumini* demonstrated the presence the flavonoids, alkaloids, glycosides, tannins, carbohydrates, amino acids etc. Phytochemicals have the complementary and overlapping mechanism of action in the body including antioxidant effects, modulation of detoxification of enzymes, stimulation of the immune system, modulation of hormone metabolism, antibacterial and antiviral effects. **Table 2** reveals ash and moisture content of *S.Cumini.*

**Fig 1** shows the DPPH radical scavenging activity of different concentrations of *S. Cumini* leaves. The $IC_{50}$ values of DPPH radical scavenging assay were found to be 93 µg/ml whereas ascorbic acid was found to be 191µg/ml. The effect of evaluating on DPPH is thought to be due to their hydrogen donating activity.

**Fig 2** illustrates the nitric oxide radical scavenging activity and it was found to have 143µg/ml whereas ascorbic acid was found to be 97µg/ml. This reaction is due to the antioxidant principle in the compound which will compete with oxygen to react with nitric oxide.

**Fig 3** shows the $IC_{50}$ values of hydrogen peroxide radical scavenging activity and was found to be 187µg/ml whereas ascorbic acid was found to be 87µg/ml. This reaction happens due to the cellular production of superoxide anion and hydrogen peroxide which favours the formation of other reactive oxygen and nitrogen species.

**Fig 4** Antimicrobial activity of *S.Cumini* leaves was evaluated against clinically significant microbes using agar well diffusion method. Methanolic leaf extract showed the considerable antibacterial activity against *L.monocytogenes, E.faecalis, C.albicans and A.niger* and the zone of inhibition was found to have 13mm, 14mm, 15mm and 15mm in 500µg respectively. As the concentration increases, *S.Cumini* is having the ability to resist against the *L.monocytogenes, E.faecalis, C.albicans and A.niger* species with comparison of standard Ciproflaxin antibiotics as positive control. As new drugs of plant origin are developed, it is appropriate to consider natural plants as a treatment for several diseases[16]. The ability of the natural medicine to influence the body systems depends on the chemical composition and antioxidant scavenging properties against free radicals.

## V. CONCLUSION

*S.Cumini* leaves is traditionally used for the treatment of various diseases and its phytochemical screening were investigated for the phytonutrients present in the *S.Cumini* leaves. Proximate analysis of the *S.Cumini* gave an idea for the chemical composition which has significant amount of carbohydrates, saponins, glycosides, total protein, moisture content and ash content. The *S.Cumini* leaves investigated were found to be a good source of phytochemicals and radical scavenging activities. *S.Cumini* has also acted upon microbial species and found to have resistant activity. Therefore, it becomes important to promote maximal use of agro by-products in the development of new functional ingredients for natural foods.

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